PATENT
Customer No. 22,852
Attorney Docket No. **02481.1693-00000**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:	)	
	)	
Habermann et al.	)	Group Art Unit: 1656
	)	
Application No.: 09/664,326	)	Examiner: Holly G. Schnizer
	)	
Filed: September 18, 2000	)	Confirmation No.: 4393
	)	
For: SIGNAL SEQUENCES FOR PREPARING LEU-HIRUDIN BY SECRETION BY *E. COLI* INTO THE CULTURE MEDIUM	)	

Attention: Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

REPLY BRIEF UNDER 37 C.F.R. § 41.41

Pursuant to 37 C.F.R. § 41.41, Appellants submit this Reply Brief in response to the Examiner's Answer mailed July 17, 2007.

Appellants do not believe that a fee is due in connection with the filing of this Reply Brief. However, if there are any fees due not enclosed herewith, please charge such fees to our Deposit Account No. 06-0916.

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I. Status of Claims

Claims 1-5 and 10-14 stand cancelled without prejudice or disclaimer. Claims 6-9 stand finally rejected. Final Office Action mailed May 30, 2006. The list of claims on appeal is attached as an appendix to this Reply Brief. (37 C.F.R. § 41.37(c)(1)(iii)). Claim 6 is the only independent claim currently pending and claims 7-9 depend from claim 6. Additionally, all amendments have been entered and considered at this time.

II. Grounds of Rejection

A. Claims 6-9

Claims 6-9 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Office argues that the claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors were in possession of the claimed invention at the time that the application was filed. Office Action dated December 29, 2005; Final Office Action dated May 30, 2006, at 2.

The Office argues that the limitation in independent claim 6 reciting that "the *E. coli* bacteria are not *E. coli* secretor mutants," added in the Amendment filed July 15, 2005, is considered new material. Office Action dated December 29, 2005, at 3.

III. Arguments

A. There is explicit support for the use of *E. coli* strains that are not secretor mutants in the methods of the invention

One of the major arguments by the Office, repeated and relied on several times throughout the Examiner's answer, is the notion that the instant specification did not disclose any "non secretor strains of *E. coli*" and failed "to suggest using a non-secretor strain in the claimed method." *See, e.g.*, Examiner's Answer at 3.

Appellants respectfully disagree. The specification clearly and unequivocally discloses that ***both*** secretor mutants ***and*** at least *E. coli* strain Mc1061 can be used in the methods of the invention. For example, the specification states:

> Competent cells of the *E. coli* strain Mc1061, or the secretor mutant WCM100, were transformed with the ligation mixture and grown under selection pressure on ampicillin-containing plates. The next morning, expression as described in Example 6 was then compared with the Ala-hirudin expression using *E. coli* strain WCM100/pCM7053. It was found that the expression obtained was about 1.5 times better than in the comparative test. (Specification at 9, lines 1-6 (underlining added)).

The underlined portion of the passage clearly indicates that Appellants were in possession of methods wherein *both E. coli* strain Mc1061 (a non secretor mutant) *and* the secretor mutant WCM100 were used. Upon reading the underlined section, one of ordinary skill in the art would understand that Appellants contemplated using *both* types of strains, secretor mutants *and E. coli* strain Mc1061 (non-secretor mutant strain). The language in the specification is unambiguous in this regard.

Therefore, contrary to the Office's opinion, the specification does disclose *E. coli* bacteria that are not *E. coli* secretor mutants.

B. Ample evidence indicates that the *E. coli* strain Mc1061 is not a secretor mutant

Another major argument by the Office, also repeated throughout the Examiner's answer, is the misconception that the instant specification did not disclose that the *E. coli* strain Mc1061 was a not a secretor mutant and that one of ordinary skill in the art would not have known such a fact.

For example, the Office states that; "Mc1061 was not identified as an *E. coli* strain that was not a secretor strain and there is nothing in the Specification to suggest that it is. One would need to lookup the characteristics of that strain to know whether or not it was a non-secreting strain." Examiner's Answer at 4 (*emphasis in original*). In another passage, the Office states that Mc1061 "is not indicated as being a non-secretor mutant and would not be recognized specifically as a non-secretor mutant by the skilled artisan." *Id.* at 6. Such statements are unsubstantiated and incorrect. Appellants are surprised that the Office is advancing this argument because, as will be shown below, one of ordinary skill in the art would readily recognize *E. coli* Mc1061 as one of the most common types of *E. coli* strains used in recombinant experiments to express a foreign protein in a bacterial host and *not* one of the secretor mutant referred to in the instant specification.

Foremost, Appellants respectfully remind the Office that "[a] patent need not teach, and preferably omits, what is well known in the art." *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *see also S3, Inc. v. Nvidia Corp.*, 259 F.3d 1364, 1371, 59 USPQ2d 1745, 1749-50 (Fed. Cir. 2001) (holding that "[t]he law is clear that patent documents need not include subject matter that is known in the field of

the invention and is in the prior art, for patents are written for persons experienced in the field of the invention.") Therefore, the specification need not disclose that Mc1061 is not a secretor mutant if one of ordinary skill in the art would know that fact. Even assuming, arguendo, that the specification does not explicitly indicate that Mc1061 is not a secretor mutant, all that is required, as the Office has correctly pointed out, is to look up the characteristics of Mc1061 to conclude that Mc1061 is not one of the secretor mutants used in the instant specification.

Secretor mutants are disclosed in the specification as having been developed and described in European Patent No. 0 448 093 (EP 0 448 093). Specification at 1, lines 6-10. Because EP 0 448 093 is in the German language, Appellants will refer to U.S. Patent No. 5,949,895 (the '895 patent) as representative of the disclosure of EP 0 448 093, since the '895 patent is a U.S. member of the patent family of EP 0 448 093. The '895 patent and EP 0 448 093 were made of record in the Information Disclosure Statement of March 30, 2001. The '895 patent defines *E. coli* secretor mutants as "*E. coli* strains which show massive protein secretion into the culture medium. A process for preparing these secretor mutants is disclosed in European Patent No. 338,410 [EP 0 338,410.]" The '895 patent at col. 3, lines 32-36. Preparation of the secretor mutants includes subjecting the *E. coli* to mutagenesis. *Id.* at col. 3, lines 41-43. As can be seen, the secretor mutants used in the instant invention could not have been know in the art prior to the priority date of EP 0 338,410, which is April of 1988. In contrast, Mc1061 was developed in 1980. *See, e.g.*, U.S. Patent No. 4, 695,542 at col. 8, lines 25-28, citing to the original journal article that first

described Mc1061. This is a clear indication that Mc1061 is not a secretor mutant because it was in existence before the secretor mutants was developed.

C. Hirudin had been expressed in non secretor mutant *E. coli* strains at the time of filing, *and was secreted* into the culture supernatant

The Office argues that "[t]he art at the time of filing had used only secretor mutants in hirudin or hirudin derivative expression." Examiner's Answer at 3. The Office also argues that "since secretory expression of the product is required by the claim and since those of skill in the art had not previously used a non-secretor strain to express hirudin or its derivatives, using a non-secretion *E. coli* strain would have been counterintuitive." *Id.*; *see also* p. 11 arguing that "the choice of secretor or non-secretor [strains of *E. coli*] is possibly essential." This misconception forms the basis of a principal argument by the Office, allegedly supporting the lack of written description for the instant claims. However, the specification discloses at least two examples of previous expression of hirudin or hirudin derivatives in non secretor mutants *E. coli* strains, which resulted in secretion of the product into the supernatant.

For example, the specification describes the experiments by Dodt et al. expressing hirudin variant HV1 in *E. coli*. Specification at 1, lines 14-18. In those experiments, 29% of the product was secreted and found in the supernatant. Secretion of a product into the supernatant is sometimes desired because purification of the product is simplified and does not involve cell lysis, which could be necessary if all of the product is retained inside the host cells. The specification also describes another instance of a group of researchers expressing and secreting a hirudin derivative, in

which 40 mg/l of product was secreted into the supernatant, compared to 300 mg/l found in the periplasm. *Id.* at 1, lines 25-28. Therefore, *E. coli* strains that are "non-secretor mutants" also have the ability to secrete the product into the supernatant. The difference with secretor mutants being one of the degree of secretion, with secretor mutants being able, in general, to secrete higher amounts of the product. It is important to note that secretion of the hirudin product into the supernatant by the non-secretor mutant *E. coli* strains mentioned above is low compared to the fraction of product that was retained in the periplasm. Increasing the amount of secreted product in the supernatant is precisely one of the goals the present invention addresses, by selecting a suitable signal sequence peptide.

As noted in the specification, even when using *E. coli* secretor mutants, secretion of leu-hirudin into the supernatant is not efficient. *See, e.g., id.* at 2, lines 9-18. Indeed, the process described in EP 0 448 093 using secretor mutants is directed to the production of ala-hirudin, which needs to be cleaved with trypsin in order to produce the desired leu-hirudin[1]. This process results in low yields of leu-hirudin. *Id.* One embodiment of the invention permits the direct expression and secretion of leu-hirudin in *E. coli*, without the need of using trypsin. *Id.* at 2, lines 19-21.

Another example of the use of *E. coli* strains that are non-secretor mutants for the expression of hirudin derivatives is present in U.S. Patent No. 5,286,714 (the '714 patent), made of record in the Information Disclosure Statement of July 15, 2005. For

[1] Leu-hirudin is the natural leech-derived substance that was found to be beneficial in anticoagulation therapy and is sold commercially under the name Refludan®. *See, e.g.*, specification at 1, lines 1-5.

example, the '714 patent indicates that a hirudin variant was "expressed in the strain *E. coli* Mc1061. Hirudin [was] isolated as in Example 5 and characterized by amino-acid analysis." The '714 patent at col. 5, lines 64-68. *See also* Example 2b of the '714 patent, which states that another hirudin variant was also "expressed in the strain *E. coli* Mc1061." *Id.* at col. 6, lines 37-38.

Therefore, the Office's argument that one of ordinary skill in the art would not have known how to express hirudin in *E. coli* strains that are non-secretor mutants, such as Mc1061, is incorrect. It also follows that because "non secretor mutants" are able to secrete the expressed product into the supernatant (albeit in low quantities) the use of secretor mutants is not a requirement in the methods of the invention, as the Office had hinted on p. 11 of the Examiner's Answer.

D. The facts from *In re Johnson* support a finding of written description support for the claim limitation "wherein the *E. coli* bacteria are not *E. coli* secretor mutants"

The Office argues that the facts from *In re Johnson* are different from those in the present case. The Office's arguments will be addressed one by one in the following remarks.

i. One of ordinary skill in the art would know that Mc1061 is a non secretor mutant

The Office states that "Mc1061 would not be recognizable as being a non-secretor strain when reading the present Specification and it is not clear that it is used in the presently claimed method." Examiner's Answer at 5.

Appellants already explained that, even if the specification does not explicitly identify Mc1061 as a non-secretor mutant, such identification is not necessary because one of ordinary skill in the art would clearly know such fact. *See* Section III.B above. Appellants also explained that the specification explicitly indicates that both Mc1061 and secretor mutants could be used in the methods of the invention. *See* Section III.A above.

ii. The specification supports the proviso "wherein the *E. coli* bacteria are not *E. coli* secretor mutants"

The Office also argues that Appellants assertion that "each of the genus-excluding provisos in *Johnson* was based on the disclosure of a single compound" is incorrect. Examiner's Answer at 6. In support of this argument, the Office cites the following passage form *In re Johnson*:

> Fifty specific choices are mentioned for the E precursor compound, a broad *class* is identified as embracing suitable choices for the E' precursor compound, and twenty-six examples are disclosed which detail fifteen species of polyarylene polyethers. Only fourteen of those species and twenty-three of the "examples" are within the scope of the claims now on appeal. Two *of the many choices* for E and E' precursor compounds are deleted from the protection sought, because appellant is claiming less than the full scope of his disclosure (emphasis added by the Office, *In re Johnson*, 558 F.2d 1008, 1018; 194 USPQ 187,195 (CCPA 1977).

The cited passage supports Appellants assertion above. *Johnson* disclosed several choices of E and E'. *Id.* However, in order to exclude the subject matter lost in an interference, *Johnson* added two provisos to the claims. The subject matter excluded by the provisos found support in only two species disclosed in the specification, one species for each proviso. *Id.* It is important to note that each proviso

excluded more species than the single species that was the basis for each proviso. *Id.* 558 F.2d at 1013; 194 USPQ at 191. Appellants arguments are directed to the fact that the appellant in *Johnson* was able to draft exclusionary provisos because the specification disclosed the excluded subject matter, even though the disclosure was of a single species and each proviso excluded subject matter broader than that single species.

The Office has already admitted that "the specification as a whole teaches that any *E. coli* strain could be used" in the methods of the invention. Final Office Action dated May 30, 2006, at bottom of page 4, underlining added. In analogy with *In re Johnson*, having described experiments where secretor mutants were used, (*see, e.g.*, the working Examples) Appellants now carve out the subject matter drawn to those secretor mutants from the pending claims. Based on *In re Johnson*, it has been recognized that inventors may claim less than the full scope of their disclosure and that "[i]f alternative elements are positively recited in the specification, they may be explicitly excluded in the claims." M.P.E.P. § 2173.05(i) (citing *In re Johnson*, 558 F.2d at 1019; 194 USPQ at 196).

iii. The specification discloses subject matter within the scope of the instant claims

The Office also argues that "[u]nlike In re Johnson, the present case does not disclose numerous species." Examiner's Answer at 6.

As mentioned before, the issue in *In re Johnson* was whether the specification supported the exclusionary provisos in the claims. In *In re Johnson*, each of the

exclusionary provisos was supported by the disclosure of a single species in the specification. Here, Appellants have described several working examples where *E. coli* secretor mutants were used and are now seeking to exclude such subject matter from the claims. Therefore, based on the ruling of In re Johnson, the proviso in instant claim 6 is supported by the original specification.

Moreover, as mentioned in Section III.A above, the specification also discloses subject matter that falls within the scope of the claims. The specification explicitly states that "[c]ompetent cells of the *E. coli* strain Mc1061, or the secretor mutant WCM100, were transformed with the ligation mixture and grown under selection pressure." Specification at 9, lines 1-6. This statement provides two options for carrying out the invention and now Appellants are limiting the claims to one of the two options (methods using non-secretor mutants). Therefore, the Office's assertion that "the present case does not have any examples of a method which meets the limitations of the claims" is incorrect.

iv. Upon reading the specification, one of ordinary skill in the art would understand that both secretor and non-secretor *E. coli* can be used in the instant invention

The Office also argues that "in an art that only has experience using secretor mutants to express hirudin derivatives in *E. coli* and in light of the claims that require secretory expression, it would not be clear to the skilled artisan that Appellant was in possession of using non-secretor strains." However, as mentioned in Section III.C above, hirudin derivatives have been expressed in non-secretor *E. coli* strains.

The Office further argues that "one of skill in the art would not recognize from the Specification that the method could be practiced with a non-secretor strain." Examiner's Answer at 8. Appellants respectfully disagree. The specification clearly indicates that the methods of the invention can be carried out using both secretor mutants and non-secretor mutants, such as Mc1061. These two options encompass the entire universe of options with respect to whether an *E. coli* strain is a secretor mutant or not. Therefore, contrary to the Office's statement, one of skill in the art would recognize that the method of the invention could be practiced with a non-secretor strain based on the instant disclosure.

In addition to the statement in the specification, one of ordinary skill in the art would understand that either *E. coli* strain (secretor mutant or non-secretor mutant) would work in the methods of the invention because the method described in claim 6 is based on a comparison of the relative secretion of hirudin or a hirudin derivative in the supernatant. Such comparison can be made when using either secretor mutants and non-secretor mutants. The signal peptide that produces the highest amount of product in the supernatant would represent the signal peptide that is most successful at expressing and secreting that given product. One of the goals of the methods instantly claimed, as the preamble of claim 6 indicates, is to select a signal peptide for secretory expression of a desired hirudin or hirudin derivative protein in *E. coli*. It is, therefore, expected that among the various signal peptides tested, some will result in lower secretion of the product into the supernatant than others. Contrast, for example, the

results of Examples 8 to 11 with the results of Examples 1 to 4 summarized in Table 2, at page 19 of the specification.

v. Appellants were in possession of the claimed methods at the time of filing

Courts have expressed the test for compliance with the statutory written description requirement as, for example, "whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at the time of the later claimed subject matter." *In re Kaslow,* 707 F.2d 1366, 1375; 217 USPQ 1089, 1096 (Fed. Cir. 1983). Applying the rule to the present case, the test would be whether Applicants were in possession of a process for selecting a signal peptide for secretory expression, wherein the *E. coli* bacteria are not *E. coli* secretor mutants. Appellants continue to assert that, at least because of the disclosure on page 9 of the specification cited above, Appellants were in possession of the claimed subject matter.

The Office also argues that "unlike In re Johnson, species excluded is not equivalent to species remaining." Examiner's Answer at 7. The Office further argues that "in the present case which is drawn to a method of expression that requires secretion, it is unclear that non-secretor mutants and secretor, mutants would function equivalently." *Id.* Appellants do not exactly understand what the Office is trying to say by stating that in *In re Johnson* the species excluded were equivalent to the species remaining. The Office did not cite to any portion of *In re Johnson* that would shed light on the Office's argument.

According to Appellants' understanding, *In re Johnson* does not require equivalency between the subject matter excluded and the subject matter remaining in the claim. On the contrary, the purpose of the provisos in *In re Johnson* was to exclude subject matter lost in an interference. *In re Johnson*, 558 F.2d at 1013;194 U.S.P.Q. at 191. If the subject matter remaining after excluding such subject matter was "equivalent" to the subject matter lost in the interference, the claims would arguably not have been allowed because they would have been within the scope of the lost count. The facts in *In re Johnson* support this view. The first proviso in *In re Johnson* excluded the species lost in the interference count. The second proviso excluded a species that was considered "'analogous' or 'equivalent'" to the first species. *Id*. Therefore, the provisos excluded the exact subject matter lost in the count *and* also "equivalent" subject matter. It follows, then, that the subject matter remaining in the claims was not "equivalent" to the excluded subject matter, or else it would have been considered to have been lost in the interference.

E. A subgenus of *E. coli* bacteria that are not *E. coli* secretor mutants is supported by the specification.

The Office further argues that Appellants citation of *In re Herschler* (591 F.2d 693; 200 USPQ 711 (CCPA 1979)) is inapposite because the "[p]resent claims do not positively recite a single species that represents an obvious class (genus) of compounds." Examiner's Answer at 9.

In support of its position, the Office advances arguments that have been addressed previously, namely that "non-secretor *E. coli* strains have not been used to

express hirudin derivatives," that "secretion is required in the present claims," and that "it would not have been known by or obvious to the skilled artisan whether or not non-secretor strains could be used to successfully practice a method of selecting signal peptide." Examiner's Answer at 10. Appellants respectfully disagree. These arguments have already been addressed in Sections III.C and III.D.iv above.

It appears the Office misunderstood the significance of *In re Herschler*. Appellants cited *In re Herschler* as an indication of the difference between providing written description support for a method claim that calls for the use of a genus of compounds (or in the instant case, a genus of bacterial strains) and a composition claim drawn to that same genus of compounds (or bacterial strains). Because the instant claims are directed to a *method* claim, Appellants are required to provide support for the method steps, but not necessarily for every single bacterial strain that can be used in the methods of the invention, as long as one of ordinary skill in the art would understand the type of strains that would work in the method of the invention.

The court in *In re Herschler* found that a single example disclosing a single corticosteroid in the solvent DSMO was sufficient written description support for a *method* of enhancing dermal penetration of a genus of "physiologically active steroid[s]." *In re Herschler*, 591 F.2d at 695; 200 USPQ at 712. The court did not require the inventor to be in possession of all possible physiologically active steroids that could be used in his method, but that he be in possession of a method of enhancing penetration of an steroid. *Id.* 591 F.2d at 701; 200 USPQ at 717. The inventor found that DMSO enhances the penetration of a number of materials through the skin. *Id.* 591 F.2d at

695; 200 USPQ at 712. The inventor then claimed a *method* comprising administration of *a physiologically active steroidal agent* and an amount of DMSO sufficient o enhance penetration of said steroidal agent. The issue faced by the court was whether *Herschler's* application was entitled to the benefit of the filing date of a great grandparent application in order to obviate rejections based on intervening prior art. *Id.* at 696. *Herschler's* great grandparent application *disclosed only one working example directed to steroids.* That example described the treatment of a patient with dexamethasone 21-phosphate (a corticosteroid). *Id.* 591 F.2d at 701; 200 USPQ at 717 Corticosteroids are a subgenus of steroids. *Id.* The court concluded that the single example disclosing dexamethasone 21-phosphate provided sufficient written description for the method comprising administration of *a genus of steroidal agents*, because, although steroids have a broad scope of physiological activity, they are chemically similar with respect to penetration of the skin aided by DMSO. *Id.* The court stated that "[i]t is not necessary that the application describe the claim limitations exactly, but only so clearly that one having ordinary skill in the pertinent art would recognize from the disclosure *that [applicants] invented processes including those limitations.*" *Id.*, emphasis added. The court summarized the issue as "would the worker of ordinary skill in this art consider 'steroidal agents' to be operative [in the method claim] when considering the great grandparent's disclosure?" *Id.* Because *Herschler's* great grandparent application adequately conveyed to one of ordinary skill in the art that steroids in general could be used in the method claimed, the court found that *Herschler* was entitled to the benefit of priority from the great grandparent application. *Id.* That is,

the court concluded that the disclosure of the single example of the enhanced penetration of the corticosteroid by DMSO was sufficient indication for one of ordinary skill in the art that steroids in general would work in the claimed method.

The equivalent inquiry in the present case would be: would the worker of ordinary skill in this art consider *E.coli* that are not secretor mutants to be operative in a process for selecting a signal peptide for secretory expression of a protein in *E. coli*, when considering the application disclosure? As mentioned before, the answer to the question is clearly affirmative. The Office has already acknowledged that "the specification as a whole teaches that *any E. coli* strain could be used and is not important to the method of the invention." Office Action dated May 30, 2006, at bottom of p. 4 (emphasis added). The specification also teaches that both secretor mutants and non-secretor mutants can be used in the claimed invention. Because the claimed method involves a comparison of expression rates using different signal peptides, one of ordinary skill in the art would understand that it is immaterial whether secretor mutants or non-secretor mutants are used in the claimed method. Therefore, the specification as a whole, including working Examples 1-12, and Table 1, clearly conveys to one of ordinary skill in the art that both *E. coli* non-secretor mutants in general, *and E. coli* secretor mutants in general are operative in the claimed methods. As with the court in *In re Herschler*, which found that steroids behave similarly with respect to DMSO-mediated penetration of the skin, expression of the signal peptide recited in the claims occurs similarly in any type of *E. coli* bacteria, whether secretor mutant or not, and,

therefore, the type of *E. coli* bacteria in which the expression of the signal peptide occurs is not essential to the claimed invention.

F. Example 18 of the USPTO training materials is analogous to the instant case

The Office indicates that Appellants' argument that Example 18 of the U.S. Patent and Trademark Office training materials (available at http://www.uspto.gov/web/menu/written.pdf) is analogous to the present situation is not persuasive. In support, the Office argues as before that "[t]he art only teaches expression of hirudin in secretion mutants" and that "[t]he Specification mentions strain Mc1061, but does not teach whether it could be used successfully in the claimed method." Examiner's Answer at 11. These arguments are without merit and have already been addressed in Sections III.C and III.D.iv above.

As mentioned in the Appeal Brief filed on February 15, 2007, the sample claim in Example 18 of the training materials is drawn to a method of expressing a protein in a particular mitochondria. The illustrative specification of Example 18 teaches the expression of a single protein using the mitochondria. The analysis section of the example indicates that, although the particular mitochondria is essential to the claimed method, a particular nucleic acid encoding for a specific protein is not. Therefore, it is not necessary to disclose all of the possible proteins that can be expressed in the mitochondria because one of ordinary skill in the art would know how to use the expression system based on the single disclosed embodiment and would recognize that Applicants were in possession of such methods.

In the instant case, as explained above, one of ordinary skill in the art would recognize from the specification that any *E. coli* bacteria, including *E. coli* that are not secretor mutants, can be used in the claimed methods. Moreover, the specific strain of *E. coli* non-secretor mutant used is not essential to the claimed methods. Therefore, one of ordinary skill in the art would recognize that Applicants were in possession of processes of selecting signal peptides wherein the *E. coli* bacteria are not *E. coli* secretor mutants.

IV. Conclusion

For the foregoing reasons, Appellants respectfully request that the Board of Patent Appeals and Interferences reverse or dismiss the outstanding rejections and allow pending claims 6-9.

Respectfully submitted,

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Dated: September 13, 2007

V. Claims Appendix

Claims on appeal:

1-5. (Canceled)—For being directed to non-elected matter.

6. (Previously presented) A process for selecting a signal peptide for secretory expression of a desired hirudin or hirudin derivative protein in *E. coli*, comprising:

(a) expressing in *E. coli* in culture medium, hirudin or a hirudin derivative which has antithrombotic activity, and which has a defined amino acid, aa_x, at its N terminus, wherein said amino acid is connected via its N-terminal to a test signal peptide;

(b) determining expression rate by measuring said hirudin or hirudin derivative activity in the culture supernatant;

(c) repeating steps (a) and (b) with various signal peptides;

(d) selecting said signal peptide by comparing the expression rates represented by the hirudin or hirudin derivative antithrombotic activity found in step (b)

wherein the *E. coli* bacteria are not *E. coli* secretor mutants.

7. (Previously presented) The process of claim 6, wherein aa_x is leucine.

8. (Previously presented) The process of claim 6, further comprising expressing said signal peptide and the desired hirudin or hirudin derivative protein in *E. coli* via a nucleic acid construct, wherein expression of the desired hirudin or hirudin derivative protein and said signal peptide occurs with simultaneous elimination of said signal peptide

wherein the *E. coli* bacteria are not *E. coli* secretor mutants.

9. (Previously presented) The process of claim 6, wherein the desired hirudin or hirudin derivative protein is hirudin.

10-14. (Canceled)—For being directed to non-elected matter.